

II. AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0020] at page 7, lines 3–22 with the following amended paragraph:

[0020] ~~Figures 2A-2D~~ Figures 2A1-2A12, 2B1-2B12, 2C1-2C4 and 2D1-2D4 show increased IFN γ production in CD8+ and CD4+ T cells elicited by HIV-1 p6 Mutant Gag vaccine. Five to seven week old Balb/c mice were injected intramuscularly with 50 μ g of either HIV-1 Gag (GagA7) or p6 mutant Gag. Some of the mice (~~Figure 2D~~ Figures 2D1-2D4) also received a Gag p42 protein boost administered intramuscularly. Five mice per test group received one injection every 2 weeks for a total of 3 DNA injections. Two weeks after the final injection, mice were sacrificed and spleens were harvested. Mice in the DNA plus protein vaccine group were given 30 ug Gag p42 protein at weeks 5 and 7 and splenocytes were removed at week 9. Splenocytes were prepared and stimulated for 6 hours with either Gag peptide pool (1 μ g/ml) or PMA (10 ng/ml) plus Ionomycin (1.6 μ M). Following stimulation, cell surface staining was carried out using rat anti-mouse FITC-conjugated anti-CD3, PerCP-conjugated anti-CD4 and APC-conjugated anti-CD8 monoclonal antibodies (BD Biosciences). Intracellular cytokine staining was carried out using rat anti-mouse PE-conjugates IFN γ , TNF α and Th2 (IL4, IL5, IL10) monoclonal antibodies followed by cytometry analysis. ~~Figures 2A and 2B~~ Figures 2A1-2A12 and 2B1-2B12 illustrate dot blot analyses (1 representative animal from the DNA only immunized groups) of CD8+ T cells producing IFN γ (Fig. 2A, upper Figures 2A1-2A6) and TNF α (Fig. 2A, lower Figures 2A7-2A12) and of CD4+ T cells producing IFN γ (Fig. 2B, upper Figures 2B1-2B6) and TNF α (Fig. 2B, lower Figures 2B7-2B12). A summary of the results from one preliminary study showing the mean levels of Th1 and Th2 cytokines relative to unstimulated controls is graphically presented for the DNA (Figure 2C Figures 2C1-2C4) and DNA plus protein (Figure 2D Figures 2D1-2D4) vaccinated mice.

Please replace paragraph [0021] at page 7, lines 23–33 with the following amended paragraph:

[0021] ~~Figures 3A-3B~~ Figures 3A1-3A6 and 3B1-3B6 show that levels of CD8+ and CD4+ T cell proliferation elicited by HIV-1 p6 Mutant Gag and GagA7 DNA vaccines are comparable. This was also found in mice vaccinated with a DNA prime plus protein

boost strategy (data not shown). Vaccination conditions, tissue harvesting and splenocyte preparation were carried out as outlined in ~~Figures 2A-2C~~ ~~Figures 2A1-2A12, 2B1-2B12 and 2C1-2C4~~. Splenocytes were prepared and labeled with CFSE (1 μ M) for 15 min at 37 C. Labeled cells were stimulated with either Gag peptide pool (1 μ g/ml) or PMA (2.5 ng/ml) plus Ionomycin (1.5 μ M) for 72 hours. Following 72 hours, cells were stained with rat anti-mouse PE-conjugated anti-CD3, PerCP-conjugated anti-CD4 and APC-conjugated anti-CD8 monoclonal antibodies (BD Biosciences) and analysed by cytometry (FACScalibur, BD BioSciences). ~~Figures 3A and 3B~~ ~~Figures 3A1-3A6 and 3B1-3B6~~ illustrate a density blot analyses of CD8+ (~~Fig. 3A~~ ~~Figures 3A1-3A6~~) and CD4+ (~~Fig. 3B~~ ~~Figures 3B1-3B6~~) T cells from representative animals of the 2 test groups.

Please replace paragraph [0125] at page 50, lines 1–21 with the following amended paragraph:

[0125] Intracellular Cytokine Staining (ICC): The levels of Th1 (IFN γ , TNF α) and Th2 (IL4, IL5 and IL10) cytokines in CD8+ and CD4+ T cells were assayed by ICC following a 5 hr stimulation with either Gag peptide pool or PMA plus Ionomycin (Iono). ~~Figures 2A and 2B~~ ~~Figures 2A1-2A12 and 2B1-2B12~~ illustrate dot blot analyses (1 representative animal from each test group) of CD8+ T cells producing IFN γ (~~Fig. 2A, upper~~ ~~Figures 2A1-2A6~~), TNF α (~~Fig. 2A, lower~~ ~~Figures 2A7-2A12~~) and of CD4+ T cells producing IFN γ (~~Fig. 2B, upper~~ ~~Figures 2B1-2B6~~), TNF α (~~Fig. 2B, lower~~ ~~Figures 2B7-2B12~~). A summary of the results from one preliminary experiment noting the mean levels of Th1 and Th2 cytokines relative to unstimulated controls are graphically represented in ~~Figure 2C~~ ~~Figures 2C1-2C4~~. As expected, Th1 and Th2 cytokine levels following PMA plus Iono stimulation did not differ significantly in either CD8+ or CD4+ T cells when comparing cells isolated from GagA7- or p6 mutant-vaccinated animals. It was found that in T cells stimulated with Gag peptide pool, IFN γ levels were augmented in both CD8+ and CD4+ T cells isolated from p6 mutant-vaccinees relative to GagA7. Neither TNF α nor Th2 cytokine levels in CD8+ and CD4+ T cells differed substantially among the two test groups. While PMA plus Iono stimulation produced comparable Th1 and Th2 cytokine levels in naïve animals as compared to that of vaccinated test animals, there was no IFN γ , TNF α or Th2 cytokines produced in response to Gag peptide pool-stimulation (data not shown). Intriguingly, IFN γ , TNF α and Th2 (IL4, IL5, IL10) cytokine levels were found to be greater in p6 mutant-

vaccinated mice relative to Gag A7, independent of the stimulation condition used. Following Gag p42 protein boost, Th1 cytokine production was also found to be greater in p6 mutant Gag vaccinated mice as compared to Gag A7 mice (Figure 2D Figures 2D1-2D4). However, this increase was not as dramatic as that noted in DNA-only immunized mice (Figure 2C Figures 2C1-2C4).

Please replace paragraph [0126] at page 50, lines 22–29 with the following amended paragraph:

[01026] Lymphocyte Proliferation: Following a 72 hour stimulation of splenocytes with Gag peptide pool or PMA plus Iono, proliferation of T cells, isolated from GagA7- and p6 mutant-vaccinated animals, was assayed using CFSE detection. Figures 3A and 3B Figures 3A1-3A6 and 3B1-3B6 illustrate a density blot analyses of CD8+ (Fig. 3A Figures 3A1-3A6) and CD4+ (Fig. 3B Figures 3B1-3B6) T cells from representative animals of the 2 test groups. There was no significant difference in the level of proliferation between the 2 test groups (Fig. 3B Figures 3B1-3B6 and data not shown). A similar result was also found in DNA plus protein vaccinated mice. The preliminary results suggest that deletion of the HIV-1 Gag P6 region does not negatively alter the level of proliferation in T cells.

III. AMENDMENTS TO THE DRAWINGS

Please delete originally submitted Figures 1, 2A-2D, 3A, 3B, 4A, 4B and 5 and substitute therefor attached Figures 1, 2A1-2A12, 2B1-2B12, 2C1-2C4, 2D1-2D4, 3A1-3A6, 3B1-3B6, 4A, 4B and 5. Attached Figures 1, 2A1-2A12, 2B1-2B12, 2C1-2C4, 2D1-2D4, 3A1-3A6, 3B1-3B6, 4A, 4B and 5 are provided on replacement sheets.

Attachments: Replacement Sheets